# Identification of Novel Human Monoclonal Antibodies to Viral Envelope Glycoproteins and Cancer-associated Antigens and Improvement of Their Efficacy

Zhang MY, Shu Y, Rudolph D, Prabakaran P, Labrijn AF, Zwick MB, Lal RB, and Dimitrov DS. Improved breadth and potency of an HIV-1-neutralizing human single-chain antibody by random mutagenesis and sequential antigen panning. *J Mol Biol* 335: 209–19, 2004.

Polyclonal antibodies have a century-old history of being effective against some viruses; recently, monoclonal antibodies (mAbs) have also shown clinical success (Dimitrov DS. *Nat Rev Microbiol* 2: 109–22, 2004). Although still the only mAb against a viral disease approved by the U.S. Food and Drug Administration (FDA), the humanized mAb palivizumab (Synagis) has been widely used for prevention of respiratory syncytial virus infections in neonates and immune-compromised individuals. Several unmodified mAbs and mAbs armed with toxins or radionuclides have received approval by the FDA for treatment of cancer; more than 400 other mAbs are in clinical trials (Waldmann TA. *Nat Med* 9: 269–77, 2003).

A fundamental problem in the development of effective therapeutic agents against viruses and cancer cells, including therapeutic antibodies, is the cells' and viruses' heterogeneity and mutability. Another problem is that antibodies that cross-react with a broad range of mutants are typically of low binding affinity: One possible solution to this is to identify highly conserved viral structures that are critical for virus entry into cells and that can serve as epitopes—the actual antigenic determinants of antigens to which antibodies bind.

To identify such conserved epitopes, we used complexes of an HIV-1 envelope glycoprotein with CD4 and coreceptor molecules for the screening of human phage libraries (Moulard M et al. *Proc Natl Acad Sci U S A* 99: 6913–8, 2002). In collaboration with Dennis Burton, PhD, of the Scripps Research Institute, a human monoclonal antibody (hmAb) Fab, X5, was identified that exhibited potent and broad neutralizing activity comparable to that of the best characterized potent broadly HIV-1–neutralizing hmAb IgG1 b12. Unlike b12, however, X5, exhibited relatively uniform neutralizing activity when tested on more than 50 primary isolates. Our collaborator Xinhua Ji, PhD, (CCR, NCI-Frederick) and his associates solved the crystal structure of X5 and found a long protruding flexible CDR3 of the antibody's heavy chain that appears to be critical for the antibody's high binding affinity; the amino acid residues forming the epitope to which X5 binds, as identified by alanine scanning mutagenesis, were highly conserved, which offers a possible explanation for X5's broad neutralizing activity (Darbha R et al. *Biochemistry* 43: 1410–7, 2004).

To further improve the binding affinity of X5 without losing its cross-reactivity, and to enhance the selection of novel broadly reactive hmAbs, we developed an approach based on sequentially changing antigens during antibody selection—termed sequential antigen panning (SAP) (Zhang MY et al. *J Immunol Methods* 283: 17–25, 2003). Several antigens representing different viral isolates were sequentially changed during the panning procedure leading to the selection of antibodies against epitopes shared among these antigens. This approach was used for the selection of several broadly cross-reactive hmAbs, including m16 (Zhang MY et al. *Antiviral Res* 61: 161–4, 2004) and m14 (Zhang MY et al. *J Virol* 78: 9233–42, 2004). To further improve the binding affinity of X5, we generated a mutant X5 library and used the SAP approach for the selection of high-affinity antibodies that bound to all antigens used for the panning and screening (Zhang MY et al. *J Mol Biol* 335: 209–19, 2004).

A single-chain hmAb selected by this procedure, m9, was extensively tested for its binding and inhibitory activity (Zhang MY et al. *J Mol Biol* 335: 209–19, 2004, and unpublished data). Its binding affinity was on average 2- to 4-fold higher with a 50-percent inhibitory concentration ($IC_{50}$) 2- to 10-fold lower than that of X5. Importantly, more primary HIV-1 isolates from different subtypes were neutralized by m9 than by X5. Thus, both the potency and breadth of neutralization were improved. m9 neutralized more than 50 primary isolates from different HIV-1 genetic subtypes including clade C, which is the dominant subtype around the world, and clade B, which is dominant in the United States. To date, only several other potent broadly HIV-1–neutralizing hmAbs (b12, 2G12, 447-52D, X5, 2F5, and 4E10/Z13) are known of the large number of antibodies tested, and it appears that m9 exhibits exceptional potency and breadth of neutralization. (For example, for a panel of 17 clade C primary isolates, it was superior to any

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other antibody tested.) NCI has filed three patent applications. Two licenses of these applications were executed with Tanox Pharmaceuticals, Inc. for m9 and several other hmAbs selected by SAP (m6, m12, m14, m16, and m18), and one license with Virosys Pharmaceuticals, Inc. for X5. More than 30 investigators from the United States, Europe, and Australia have requested and received them.

The identification and characterization of novel broadly neutralizing hmAbs and their epitopes could also help in the development of vaccine immunogens that could elicit the same antibodies *in vivo*. This approach, termed retrovaccinology (Burton DR. *Nat Rev Immunol* 2: 706–13, 2002), contrasts with standard approaches based on evaluation of the antigen first. We found that some mutations in the HIV-1 envelope glycoproteins significantly increased binding of the antibodies we identified; these mutated glycoproteins may have potential as vaccine immunogens. A large amount of work based on a variety of antigens failed to elicit *in vivo* any of the few known potent broadly HIV-1–neutralizing antibodies; perhaps such mutated envelope glycoproteins could help solve this problem that is of major importance for vaccine development.

A number of similarities exist in the strategies used by cancer cells and viruses causing chronic diseases (e.g., HIV) regarding the evasion of immune responses as well as in the mechanisms of the ligand-receptor interactions leading to virus entry and signal transduction across membranes. We are currently developing hmAbs against components of the insulin-like growth factor (IGF) system using approaches similar to those we have developed for anti–HIV-1 hmAbs. We have recently identified two high-affinity hmAbs against IGF-II and, in collaboration with J. Carl Barrett, PhD, (CCR, NCI) and his associates, are characterizing them as potential candidate cancer therapeutics.

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## ■ TRANSLATIONAL RESEARCH

# Dual Role of Transforming Growth Factor-β Signaling in Breast Cancer

Tian F, DaCosta Byfield S, Parks WT, Yoo S, Felici A, Tang B, Piek E, Wakefield LM, and Roberts AB. Reduction in Smad2/3 signaling enhances tumorigenesis but suppresses metastasis of breast cancer cell lines. *Cancer Res* 63: 8284–92, 2003.

The compound transforming growth factor-β (TGF-β) received its name due to its ability to transform normal fibroblasts by inducing their anchorage-independent growth. This *in vitro* "transforming" or presumed pro-oncogenic activity of TGF-β is now known to predominate only in late stages of carcinogenesis, manifesting itself as a pro-metastatic activity and involving (1) reduction of cell adhesion (sometimes associated with epithelial-to-mesenchymal transition [EMT]), (2) increased cell mobility, and (3) increased production of matrix-degrading proteins such as the metalloproteinases, typically associated with invasive activity. Paradoxically, TGF-β also has potent tumor suppressor activity, based in part on its ability to inhibit the growth of most epithelial and lymphoid cells, which form the basis of most human cancers. This activity is now thought to predominate in the earlier stages of cancer progression, stages during which the cells remain sensitive to the growth inhibitory effects of TGF-β, having not yet reached the point of unchecked proliferation.

The unanswered question is how TGF-β might switch from a tumor suppressor to a pro-metastatic agent. An important, known fact is that the expression levels of the TGF-β transmembrane receptors are reduced as cells progress from early to late stages of carcinogenesis. Consistent with this reduction in receptor levels, end points such as growth inhibition, which are known to require robust signaling over a period of at least 8 to 10 hours, cannot be sustained. In addition to changes in signaling strength and duration, it is also important to know the extent to which the signaling context is changed as cells progress from a premalignant state to one in which they are fully invasive and metastatic.

TGF-β acts via unique transmembrane receptor serine/threonine kinases. Signals from these receptors are transduced primarily via a family of latent transcription factors called Smad proteins. In the case of TGF-β, Smad2 and Smad3 are direct substrates of the type I receptor kinase, being activated by phosphorylation on a C-terminal serine motif. Once activated, they partner with the common mediator Smad4 and are translocated to the nucleus where they regulate transcription of target genes in collaboration with a wide variety of sequence-specific transcription factors. Not only may the balance between Smad2 and Smad3 signaling change during the course of carcinogenic transformation of cells, but the balance between Smad signaling and that of other pathways activated by TGF-β, such as the mitogen-activated protein kinase (MAPK) or the phosphatidylinositol 3-kinase (PI3K) signal transduction pathways, may also change, such that a shift in pathway utilization may occur as TGF-β changes from tumor suppressor to pro-metastatic activity (Figure 1).

To address these issues, we have used a set of cells derived from Ras-transformants